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Synthesis and characteristics of polyhydroxy triglycerides from milkweed oil

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Abstract

The milkweed family *Asclepiadaceae* comprises many genera including the genus *Asclepias syriaca*, otherwise known as the common milkweed. This plant had been considered a nuisance and serious efforts made toward its eradication. However, milkweed has become an industrial crop of growing significance on account of market demand for its hypoallergenic floss in pillows, comforters and other industrial uses. Processing of milkweed pods gives three product streams of floss, seed and pod hulls. The seed contains about 25% by weight of very highly unsaturated oil with some unusual fatty acids. The objective of this study was to generate value-added products from milkweed oil. To achieve this, the triglycerides of *A. syriaca* seed were oxidized to the polyoxirane and polyhydroxy triglyceride derivatives by means of an in situ peroxy acid method. The epoxy triglycerides produced exhibited high stability and highly viscous behavior, whereas the polyhydroxy triglycerides showed additional unusually stable emulsifying properties for oil in water emulsions. Published by Elsevier Science B.V.

Keywords: *Asclepias syriaca*; Milkweed triglycerides; Peroxy acid; Epoxy triglyceride; Polyhydroxyl triglyceride; Stable emulsion

1. Introduction

The milkweed family Asclepiadaceae comprises some 200 genera and 2500 species with a wide ranging habitat from southern Canada to northern Mexico and throughout the United States. The common milkweed species (*Asclepias syriaca* L.), grows mainly between the Rocky Mountains and Appalachia of the United States and is considered a pest by most farmers. However, milkweed is now an industrial crop. The pods generate three product types, a fine silky

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[1] Names are necessary to report factually on available data: however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1
Fatty acid composition of milkweed (*A. syriaca*) oil

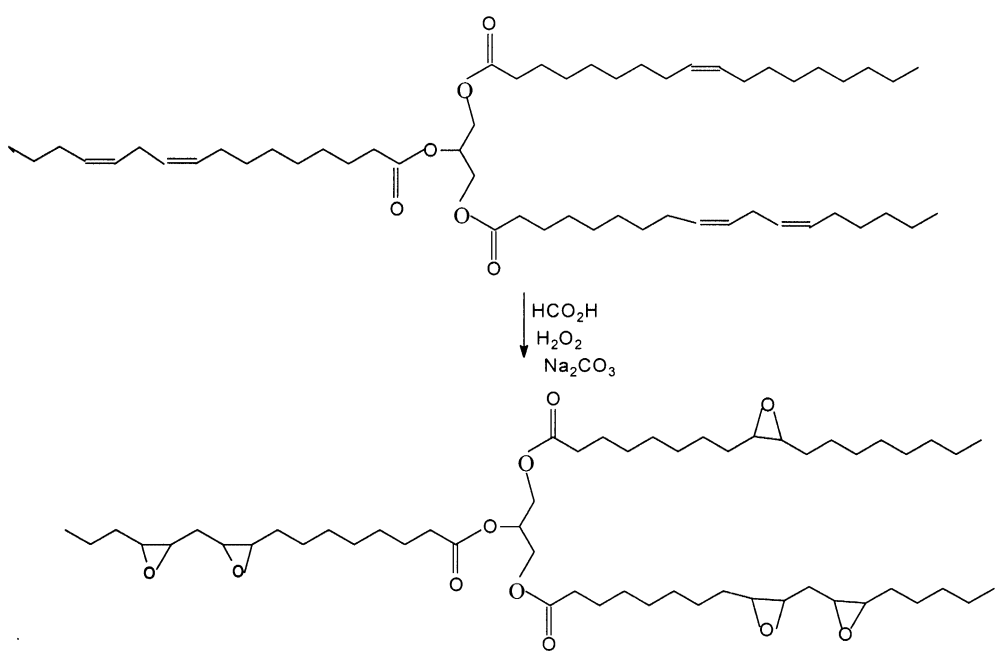
Acid type	% Content
Oleic	31.0 (Δ^9, Δ^{11})
Linoleic	50.5
Linolenic	1.2
Palmitoleic	9.6 (Δ^9, $\Delta^{9,12}$)
Palmitic	5.7
Stearic	2.5

Phillips et al., 1996.

fiber, seed and pod hulls. The fiber is currently being used in pillows and comforters.

The seed has about 25% oil content. The oil-free seed meal is a potent nematicide and pesticide against army worms (Harry-O'kuru et al., 1999). Even the pod hulls have the bioactive nematicide action. Milkweed oil has been shown to be highly unsaturated (Phillips et al., 1996), Table 1, and cardenolide-free (Harry-O'kuru and Abbott, 1997). At present, this vegetable oil cannot successfully compete as an edible oil because of its low cultivated acreage. It could do better in a low-volume, value-added niche market. Apart from the many uses in the food industry, vegetable oils as renewable resources, have not been fully utilized in industry. This shortcoming is due in part to the limited functional groups present in the triglyceride molecules of the traditionally available vegetable oils. In contrast, castor oil with a single hydroxyl group per ricinoleic acid moiety in the triglyceride structure has a variety of industrial applications.

The advent of unique new crop oils bearing epoxy groups (*Vernonia galamensis* seed, Ayorinde et al., 1990) or hydroxyl triglycerides (*Lesquerella fendleri*, Lesquerella Task Force, 1991) will change this situation for vegetable oils. Vegetable oils from commodity crops such as soybeans and corn and their unsaturated fatty acid methyl esters have been additionally functionalized using a variety of processes (chemical, enzymatic or a combination of both) to provide oil components with properties amenable to food and non-food industrial applications (Schmitz and Wallace, 1954; Chadwick et al., 1958; Rusling et al., 1968;



Scheme 1.

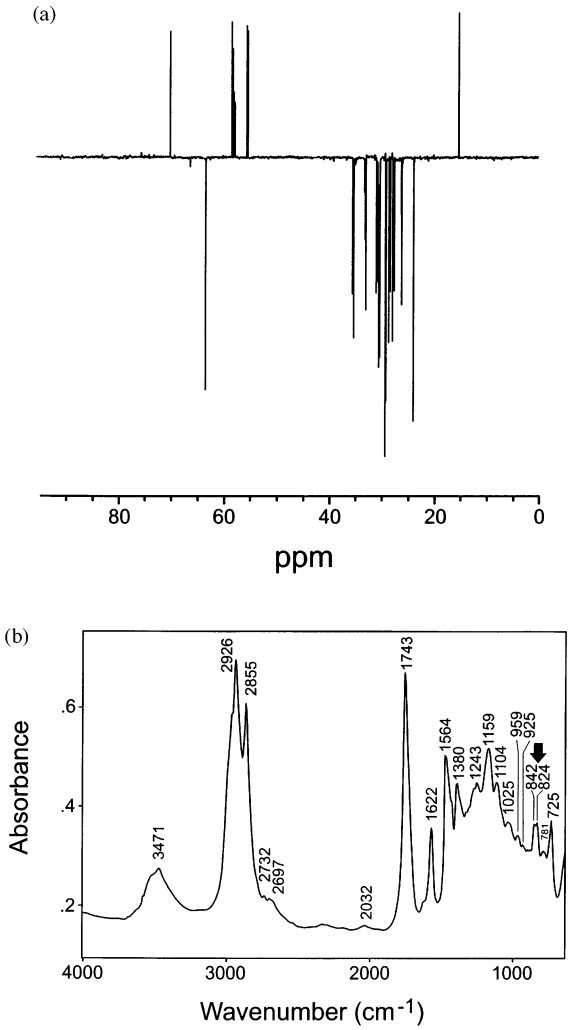


Fig. 1. (a) ^{13}C NMR spectrum (DEPT) of the tetra-epoxy triglyceride of refined *A. syriaca* (the common milkweed) oil; (b) FTIR spectrum of the tetra-epoxy triglyceride of the refined *A. syriaca* oil.

Meffert, 1984; Barrett et al., 1993; Dahlke et al., 1995; Buisman et al., 1998). In this manuscript, we report a chemical modification of milkweed oil in a one-pot process to generate higher quality, value-added base products (epoxy triglycerides and polyhydroxyl triglycerides) for non-food applications.

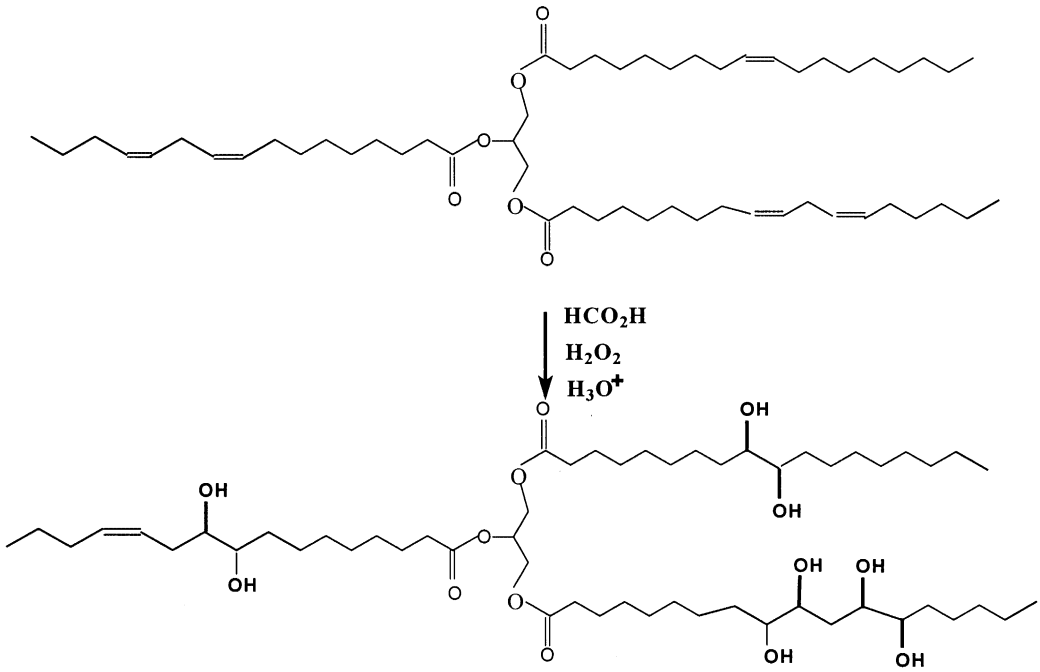
2. Materials and methods

2.1. Materials

Crude, cold-pressed milkweed oil was obtained from Natural Fibers Corporation (Ogallala, NE). Activated acid clay (Bentonite) was obtained from Harshaw/Filtrol Clay Products Division, (Jackson, MS). Sample centrifugation was performed using a Beckman Coulter centrifuge, model J2-HS (Beckman Coulter, Inc., Fullerton, CA). Formic acid, (90.4 and 96%) was from Fisher Scientific, (Chicago, IL) and hydrogen peroxide 50% in water, was from Aldrich Chemical Company, (St. Louis, MO). FTIR spectra were recorded on a Bomem MB-Series FTIR, (Bomem Québec, Canada) and ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker ARX-400 with a 5 mm dual proton/carbon probe (Bruker Spectrospin, Ballerica, MA). Specific rotation $[\alpha]_D^{20}$ values were measured on a Perkin-Elmer Polarimeter Model 341 (Perkin-Elmer, Norwalk, CT). Kinematic viscosities were measured on a Cannon viscometer with the 400 (378E) or 300 tubes in a Temp-Trol Viscosity Bath, (Precision Scientific, Chicago, IL).

2.2. Methods

Expelled milkweed oil: Press oil was obtained by pressing whole seed with a pilot-scale Hander expeller, (Hander Oil Machinery Corp., Osaka, Japan). Approximately 120 kg of milkweed seed was pressed at a feed rate of 40 kg h^{-1}. An initial portion of seed was recycled through the press until the press temperature reached 80 °C. The choke was adjusted to minimize the generation of fine particulates while maintaining a barrel temperature between 80 and 90 °C. Meal from the first press was collected and sent through the press a second time. The crude oil was centrifuged with a pilot-scale centrifuge (Model AS-16P Super Centrifuge, The Sharples Corp., Philadelphia, PA) to remove residual fines and stored under nitrogen. The crude (dark-green) oil was refined by mixing with 5–10 (wt.%) of acid-activated Harshaw/Filtrol clays and either vacuum filtered or centrifuged



Scheme 2.

at 10×1000 *g* for 15 min. The clear, light yellow filtrate or supernatant centrifugate was carefully decanted and used as described subsequently.

2.3. Synthesis of epoxy triglyceride

In a typical process, reprocessed milkweed oil (582.0 g, 673.76 mmol), iodine value = 111.4, $[\alpha]_D^{20} = 0.11°$ (*c* 0.06, CH_2Cl_2) was placed in a 1 l, three-necked jacketed flask equipped with a mechanical stirrer and was heated to 45.5 °C. Formic acid (96%, 39.7 g, 0.3 equiv./mol of C═C) was added and the mixture stirred to homogeneity. Hydrogen peroxide (50%, 320 ml, 6.74 mol) was then added slowly (i.e. dropwise). At the end of hydrogen peroxide addition, the temperature was raised to 70 °C and vigorous stirring was continued for 7 h. The heat source was then removed, the reaction mixture allowed to cool and the mixture transferred to a separatory funnel with ethyl acetate as diluent. The material was washed with saturated NaCl (300 ml × 4) followed by saturated Na_2CO_3 (40 ml) in additional NaCl solution. When a pH of 7.5 was reached, the organic phase was then washed with deionized water. The wet organic layer was separated from a turbid aqueous phase and was concentrated at 60 °C in vaccuo to remove the

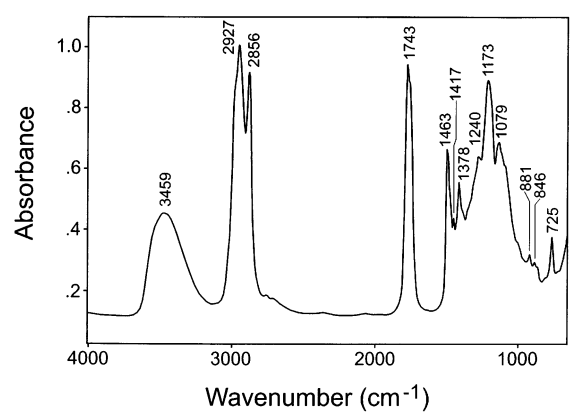


Fig. 2. FTIR spectrum of the polyhydroxy triglyceride of *A. syriaca* oil.

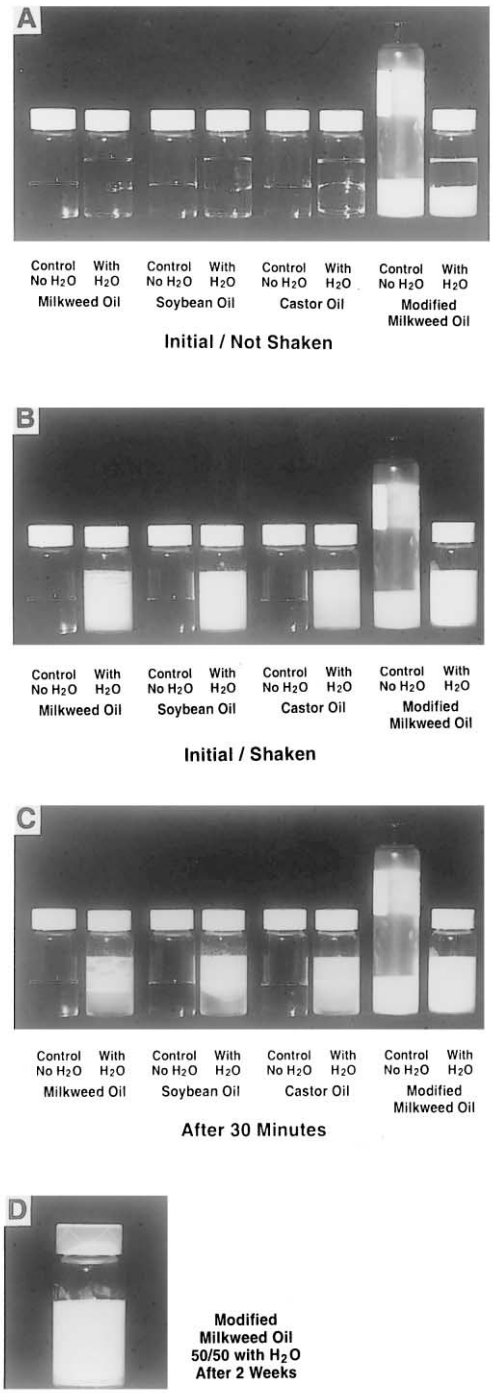


Fig. 3.

solvent and water. The yield of epoxy triglyceride was 558.4 g; the measured kinematic viscosities were: 1208.95 centistokes (cs) at 40 °C and 81.3 cs at 100 °C. PV = 9.4, IV = 1.79. The specific rotation was $[\alpha]_D^{20} = +0.17°$. An aqueous fraction (42.0 g) was reclaimed from the final water-wash following concentration at 70 °C, to give a total yield of 600.6 g (97%). IR values were (film on KBr, main fraction) cm^{-1}: 3471 w, 2927 vs, 2856 vs, 1743 vs, 1560 w, 1463 s, 1375 s, 1242 s, 1162 s, 1105 s, 1048 s, 845–824 d(m), 726 w-m. IR (minor fraction) cm^{-1}: 3455 s, 2926 vs, 2855 s, 1743 vs, 1560 w, 1463 m, 1380 m, 1255 m, 1162 m-s, 1106 m, 824–842 *d* (w), 725 d(w-m). ^{1}H-NMR (main fraction in $CDCl_3$) δ: 5.25 m (residual vinylic), 4.29 dd ($J = 4.3$, 11.9 Hz, 2H), 4.14 dd ($J = 5.9$, 11.9 Hz, 2H), 3.1 m (2H), 2.96 m (2H), 2.89 m (2H), 2.3 m (6H), 1.75–1.25 m (72H), 0.87 m (9H). ^{13}C ($CDCl_3$) δ: 173.1, 172.7, 68.87, 62.02, 57.10, 57.05, 56.91, 56.85, 56.63, 56.55, 54.25, 54.09, 34.06 , 33.90, 31.79, 31.61, 29.63, 29.47, 29.28, 29.23, 29.1 5, 29.12, 28.92, 28.88, 27.83, 27.77, 27.75, 27.16, 26. 88, 26.55, 26.52, 26.08, 24.73, 22.51, 13.93.

2.4. Synthesis of polyhydroxy triglycerides

Reprocessed milkweed oil (648.0 g, 759.9 mmol) in a 1 l three-necked jacketed flask was stirred vigorously at 40 °C and formic acid (90.4%, 62.2 g, 1.22 mol) was added in one portion followed with a slow (dropwise) addition of H_2O_2 (50%, 203.0 g, 2.98 mol). At the end of peroxide addition, the temperature was increased to 70 °C. After 15 h, the heat source was removed and stirring was continued, to allow the reaction mixture to cool to room temperature after which the aqueous phase was removed.

Fig. 3. (A) Four pairs of samples are shown: thefirst vial in each pair is the control compound, whereas the second vial in each set has an equal mass of water carefully added to the sample so as to form two distinct layers; (B) A picture of the samples taken immediately after 2 min of thorough manual mixing of the second vial in each sample pair; (C) Appearance of the emulsions 30 min after the initial mixing; (D) Appearance of the emulsion formed by the polyhydroxy triglyceride (modified milkweed oil) after 2 weeks of the initial mixing.

Deionized water (300 ml) was added and followed with 6 M HCl (100 ml). The nearly colorless sludge was stirred at 70 °C overnight. The cream-colored product was transferred into a separatory funnel using ethyl acetate as diluent. The aqueous layer was discarded and the organic phase washed sequentially with saturated NaCl solution, saturated $NaHCO_3$ to a pH of 7.5, and deionized water. Ethanol was added to facilitate separation of the phases. After removal of the aqueous layer, the product was vacuum concentrated at 70 °C. The yield was 711.60 g (94.7%) of the polyhydroxyl triglyceride with an oxirane value = 1.35; iodine value = 14 compared to an iodine value of 111.4 in the starting milkweed oil. The measured kinematic viscosities were: 2332.5 centistokes at 40 °C and 75.53 centistokes at 100 °C. Specific rotation $[\alpha]_D^{20} = +0.37°$. IR (film on KBr) cm^{-1}: 3636–3168 b, 2927 vs, 2856 vs, 1743 vs, 1463 s, 1378 m-s, 1240 m-s, 1173 vs, 1097 s, 881 w, 725 w-m. ^{13}C-NMR ($CDCl_3$) δ: 173.2, 172.8, 84.60, 83.00, 82.50, 82.00, 80.50, 74.40, 73.82, 73.20, 68.82, 62.04, 34.75, 34.48, 34.10, 33.94, 33.54, 31.79, 31.61, 30.47, 29.64, 29.56, 29.47, 29.42, 29.31, 29.26, 29.21, 29.17, 29.05, 28.91, 25.58, 25.26, 24.76, 22.61, 22.56, 22.45, 14.08.

3. Results and discussion

3.1. Epoxy triglyceride

The oxidation of olefinic bonds of unsaturated fatty acids and their methyl esters using organic peracids, that is, the Prileschajew reaction has been intensely studied and reviewed (Findley et al., 1945; Barrett et al., 1993) because of the latent potential of triglycerides as renewable industrial feedstock from vegetable oils. During the exploratory stage of this study to determine methods suitable to milkweed oil oxidation, we investigated several methods including the chemoenzymic approach (Rusch gen Klaas and Warwel, 1996; Frykman and Isbell, 1997). Although these processes were moderately successful without optimization, an alternative and cost-effective oxidation system was sought that would more satisfactorily address the special needs of milkweed oil. Thus, in a modification of the in situ performic acid oxidation method reported by Chadwick et al. (1958), Page-Xatart-Pares et al. (2000), the oxidation of refined milkweed triglycerides resulted in almost quantitative yields of the oxirane triglycerides when 0.4 equivalent of formic acid and 3.0 mol of H_2O_2 per mol of carbon–carbon double bond were employed under neat reaction conditions, Scheme 1. Its ^{13}C-NMR chemical shifts data show eight carbon resonances, 57.01, 57.05, 56.91, 56.85, 56.63, 56.55, 54.25 and 54.09 ppm, ascribable to the epoxy ring carbons, Fig. 1a. Because epoxidation is a stereospecific addition reaction to the C–C double bonds which have *cis*-configuration in the natural triglyceride, the reaction product is the *cis* tetra-oxirane compound based on the data. This is consistent with published literature (Gunstone, 1993).

Two other ^{13}C resonances worth mentioning here are the primary C1, C3 of the esterified glycerol moiety that are chemically and magnetically equivalent and therefore overlap at 62.02 ppm while its C2 is observed at 68.87 ppm. Although the ^{13}C of this product gave no resonances corresponding to olefinic carbons, the ^{1}H spectrum of the same sample does indicate some residual vinylic proton(s) suggesting that the product was not completely epoxidized. This is not surprising on account of the level of unsaturation in the starting triglyceride.

The *Fourier Transform-IR* (FTIR) spectrum of this product, Fig. 1b, shows the characteristic doublet at 824–842 cm^{-1} of the C–O–C oxirane stretch usually observed in the naturally epoxidized vegetable oils (Ayorinde et al., 1990). An inherent feature of peracid epoxidation, however, is the intrinsic acid strength of the acid which tends to cause some oxirane ring-opening to the diols as previously noted by Findley et al. (1945), in acetic acid solvents and Page-Xatart-Pares et al. (2000), using performic acid.

In our circumstance the epoxy ring-opening was not a serious drawback since our target product was the polyhydroxy triglycerides.

When the epoxide is the sole product desired, the reaction conditions and progress could be strictly timed and monitored so as to be quenched at the optimum moment with the least amount of diol formation. The physical properties of the tetra-oxirane triglycerides include a high kinematic viscosity 1209 centistokes; an epoxide value of 9.4 and an iodine value of 1.79 compared to 111.4 for the parent oil.

3.2. Polyhydroxy triglyceride

The polyhydroxy triglyceride was synthesized in a single step, that is, without pre-separation of the intermediate epoxide, Scheme 2. The process is high-yielding, giving a material of unique properties. The presence of several pendant hydroxyl groups gives this compound the ability to form inter- and intramolecular hydrogen-bonds. Besides hydrogen-bonding, the pendant substituents on the main chains impart tacky behavior to the molecules as they entangle with each other. These characteristics manifest a very high kinematic viscosity (2332 centistokes at 40 °C and 75.5 centistokes at 100 °C). The much lower viscosity observed at 100 °C confirms the role played by hydrogen-bonding in the behavior of this compound.

The IR spectral features, Fig. 2, of this compound are the broad O–H stretching mode at 3168–3636 cm^{-1}, a strong ester carbonyl band at 1743 cm^{-1} and the disappearance of the H–C = stretch and C=C breathing modes of the olefin starting material. Acetylation of the hydroxyl functions (not described above) gave a much cleaner proton and carbon spectra showing better line resolution. The use of the distortionless enhancement polarization transfer (DEPT) experiment in the spectrum of the acetylated compound enabled resolution of the two primary glycerol C1, C3 at 62.02 and 65.78 ppm, respectively. The tertiary carbons bearing the acetylated hydroxyls, thus are lower field at 63.64, 70.05, 73.42, 73.79, 75.03, 77.50, 81.02.

One of the interestingly important characteristics of the polyhydroxy triglyceride is its moisture holding capacity. The compound forms very stable emulsions, when a 50/50 wt.% mixture of it in water is agitated, Fig. 3A–D. Transparency of the polyhydroxyl triglyceride (modified milkweed oil) was later obtained after solubilization in organic solvent, followed with desiccation to remove occluded residual water, not otherwise removable at 70 °C under vacuum.

4. Conclusions

Highly unsaturated milkweed oil is readily and conveniently convertible into epoxy and polyhydroxy triglycerides using currently available industrial techniques. These converted products exhibit properties that can be used in many industrial applications. Thus, the milkweed plant instead of being perceived as a problem is rather a useful crop that could help the rural farm economy.

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